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In the Claims

Please amend the claims as follows:

- 1. (Original) A vector comprising a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector, once digested with the first and second restriction enzymes and ligated to a DNA fragment comprising an open reading frame flanked by an end generated by SgfI and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, yields a recombinant vector comprising the open reading frame.
- 2. (Original) The vector of claim 1 wherein the second and third restriction enzymes are the same.
- 3. (Original) The vector of claim 1 wherein the second and third restriction enzymes are different.
- 4. (Original) The vector of claim 1 wherein the second restriction enzyme is *PmeI*, *EcoRV* or *BalI*.
- 5. (Original) The vector of claim 1 wherein the second restriction enzyme is *PmeI*, *DraI*, *EsaBC3I*, *HindIII*, *HpaI*, *SciI* or *SwaI*.

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6. (Original) The vector of claim 1 wherein the second restriction enzyme is AluI, BalI, BfrBI, BsaAI, BsaBI, BsrBI, BtrI, Cac8I, CdiI, CviJI, CviRI, Eco47III, Eco78I, EcoICRI, EcoRV, FnuDII, FspAI, Hael, HaeIII, Hpy8I, LpnI, MlyI, MslI, MstI, NaeI, NalIV, NruI, NspBII, OliI, PmaCI, PmeI, PshAI, PsiI, PvuII, RsaI, ScaI, SmaI, SnaBI, SrfI, SspI, SspD5I, StuI, XcaI, XmnI, or ZraI.

- 7. (Original) The vector of claim 1 wherein the restriction enzyme that generates a 3' TA overhang is SgfI.
- 8. (Original) The vector of claim 1 which further comprises an open reading frame which includes the recognition site for the first restriction enzyme.
- 9. (Original) The vector of claim 1 which comprises an appropriately positioned ribosome binding site 5' to the nucleotide cleaved by the first restriction enzyme.
- 10. (Currently amended) The vector of claim 1 wherein ligation generates the following sequence in the recombinant vector AAGGAGCGATCGCYATG (SEQ ID NO:69) or $X_1X_2X_3GCGATCGCCATG$ (SEQ ID NO:70), wherein X_1-X_3 , X_2X_3G or X_3GC is a codon which is not a stop codon, and wherein Y is A, T, G or C.
- 11. (Original) The vector of claim 1 wherein ligation generates the following sequence in the recombinant vector $X_1X_2X_3GTTTY_1Y_2$, wherein $X_1X_2X_3$ is a codon in an open reading frame which is not a stop codon and Y_1 and Y_2 each =A, Y_1 = A and Y_2 = G or Y_1 = G and Y_2 = A.

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12. (Original) The vector of claim 1 wherein ligation generates the following sequence in the

recombinant vector $X_1X_2X_3GTTTY_1Y_2$, wherein $X_1X_2X_3$, X_2X_3G or X_3GT is a codon in an open

reading frame which is not a stop codon and Y₁ is not A when Y₂ is A or G, or Y₁ is not G when

 Y_2 is A.

13. (Original) A vector comprising a first open reading frame which includes a recognition

site for a first restriction enzyme that generates a 3' TA overhang and a recognition site for a

second restriction enzyme that is not in the open reading frame generates blunt ends, which

vector, once digested with the first and second restriction enzymes and ligated to a DNA

fragment comprising a second open reading flanked by an end generated by Sgfl and a third

restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from

at least one species and generates blunt ends, yields a recombinant vector comprising a third open

reading frame comprising the first and second open reading frames, which third open reading

frame encodes a fusion peptide or protein.

14. (Currently amended) A vector comprising a ribosome binding site which optionally

overlaps by one nucleotide with a SgfI recognition site and a recognition site for a first restriction

enzyme that generates blunt ends, which vector, once digested with SgfI and the first restriction

enzyme and ligated to a DNA fragment comprising an open reading frame encoding a peptide or

polypeptide flanked by

5' CGCCATGX₁Y₁

3' TAGCGGTACX₂Y₂

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and a blunt end generated by a second restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, yields a recombinant vector which encodes the peptide or polypeptide, wherein X_1 is the first codon which is 3' to the start codon for the open reading frame, wherein X_2 is the complement of X_1 . wherein Y_1 is the remainder of the open reading frame, and wherein Y_2 is the complement of Y_1 .

15. (Original) A support comprising a plurality of recombinant vectors, two or more of which comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a first restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by SgfI and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

16. (Original) The support of claim 15 wherein the vector further comprises a second open reading frame 3' to the promoter which second open reading frame includes the recognition site for the first restriction enzyme, which second open reading frame, when ligated to the first open reading frame, forms a third open reading frame which encodes a fusion peptide or protein.

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17. (Currently amended) The support of claim 15 wherein ligation generates the following sequence in the recombinant vector AAGGAGCGATCGCYATG (SEQ ID NO:69) or $X_1X_2X_3$ GCGATCGCCATG (SEQ ID NO:70), wherein X_1 - X_3 , X_2X_3 G or X_3 GC is a codon which is not a stop codon, and wherein Y is A, T, G or C.

- 18. (Original) The support of claim 15 wherein ligation generates the following sequence in the recombinant vector $X_1X_2X_3GTTTY_1Y_2$, wherein $X_1X_2X_3$ is a codon in an open reading frame which is not a stop codon and Y_1 and Y_2 each =A, Y_1 = A and Y_2 = G or Y_1 = G and Y_2 = A.
- 19. (Original) The support of claim 15 wherein ligation generates the following sequence in the recombinant vector $X_1X_2X_3GTTTY_1Y_2$, wherein $X_1X_2X_3$, X_2X_3G or X_3GT is a codon in an open reading frame which is not a stop codon and Y_1 is not A when Y_2 is A or G, or Y_1 is not G when Y_2 is A.
- 20. (Original) A support comprising a plurality of recombinant vectors, two or more of which comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame comprising a second open reading frame and one or more codons which are in-frame with the second open reading frame, wherein the second open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of
- a DNA sequence comprising the second open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that

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generates complementary single-strand DNA overhangs, which DNA sequence is digested with

Pmel and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame

codons and the promoter which is 5' to an end generated by a second restriction enzyme which

generates single-strand DNA overhangs which are complementary to the single-strand DNA

overhangs generated by the first restriction enzyme.

21. (Original) The support of claim 20 wherein the exchange site formed by blunt end

ligation includes N₁N₂N₃GTTTN₄N₅, wherein N₁N₂N₃GTTT is a sequence from the 3' end of the

DNA sequence, wherein if $N_1N_2N_3$ do not code for a stop codon, N_4 and $N_5 = A$, or $N_4 = A$ and

 $N_5 = G$ or $N_4 = G$ and $N_5 = A$, or wherein $N_1N_2N_3$ code for a stop codon.

22. (Original) A support comprising a plurality of recombinant vectors, two or more of which

comprise an open reading frame for a different polypeptide, wherein at least one recombinant

vector comprises a promoter and an open reading frame which is flanked by two exchange sites,

wherein the exchange sites are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two

restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction

enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first

DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs,

and

a vector comprising the promoter and non-essential DNA sequences that are flanked by

two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic

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restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

- 23. (Original) The support of any one of claims 15 or 20 to 22 which is multi-well plate.
- 24. (Original) The support of any one of claims 15 or 20 to 22 wherein the plurality of recombinant vectors each encode a different polypeptide from the same organism.
- 25. (Original) The support of any one of claims 15 or 20 to 22 wherein the plurality of recombinant vectors encode orthologous polypeptides.
- 26. (Original) The support of any one of claims 15 or 20 to 22 wherein the plurality of recombinant vectors encode paralogous polypeptides.
- 27. (Original) A method to prepare a support comprising a plurality of recombinant vectors or recombinant cells, comprising:
- a) selecting a plurality of recombinant vectors or recombinant cells comprising recombinant vectors, wherein two or more of the recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame which is flanked by two exchange sites, wherein the exchange

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sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang, which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction

enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by

SgfI and an end generated by a third restriction enzyme which has infrequent restriction sites in

cDNAs or open reading frames from at least one species and generates blunt ends; and

b) introducing the selected recombinant vectors or recombinant cells to one or more

receptacles of the support.

28. (Original) A method to prepare a support comprising a plurality of recombinant vectors or

recombinant cells, comprising:

a) selecting a plurality of recombinant vectors or recombinant cells comprising

recombinant vectors, wherein two or more of the recombinant vectors comprise an open reading

frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter

and a first open reading frame comprising a second open reading frame and one or more codons

which are in-frame with the second open reading frame, wherein the second open reading frame

is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the second open reading frame which includes a PmeI

recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that

generates complementary single-strand DNA overhangs, which DNA sequence is digested with

PmeI and the first restriction enzyme, and

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a vector comprising a blunt end at the 5' end which is 5' to the one or more codons and

the promoter which is 5' to an end generated by a second restriction enzyme which generates

single-strand DNA overhangs which are complementary to the single-strand DNA overhangs

generated by the first restriction enzyme; and

b) introducing the selected recombinant vectors or recombinant cells to one or more

receptacles of the support.

29. (Original) A method to prepare a support comprising a plurality of recombinant vectors or

recombinant cells, comprising:

a) selecting a plurality of recombinant vectors or recombinant cells comprising

recombinant vectors, wherein two or more of the recombinant vectors comprise an open reading

frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter

and an open reading frame which is flanked by two exchange sites, wherein the exchange sites

are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two

restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction

enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first

DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs,

and

a vector comprising the promoter and non-essential DNA sequences that are flanked by

two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic

restriction enzyme, which vector is digested with the second restriction enzyme to generate a

second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair

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of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the

non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA

overhangs of the first pair of non-self complementary single-strand DNA overhangs; and

b) introducing the selected recombinant vectors or recombinant cells to one or more

receptacles of the support.

30. (Original) The method of any one of claims 27 to 29 wherein each of the selected

recombinant vectors encodes a different paralogous protein.

31. (Original) The method of any one of claims 27 to 29 wherein each of the selected

recombinant vectors encodes a different protein in a catabolic pathway.

32. (Original) The method of any one of claims 27 to 29 wherein each of the selected

recombinant vectors encodes a different protein in a biosynthetic pathway.

33. (Original) The method of any one of claims 27 to 29 wherein each of the selected

recombinant vectors encodes a different protease.

34. (Original) The method of any one of claims 27 to 29 wherein each of the selected

recombinant vectors encodes a protein from the same organism.

35. (Original) The method of any one of claims 27 to 29 wherein each of the selected

recombinant vectors encodes orthologous proteins.

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36. (Original) A method to prepare a plurality of mutagenized recombinant vectors, comprising:

a) providing DNAs comprising a plurality of mutagenized open reading frames flanked

by a SgfI recognition site and a site for a first restriction enzyme which has infrequent

restriction sites in cDNAs or open reading frames from at least one species and generates

blunt ends; and

b) digesting the DNAs with SgfI and the first restriction enzyme and ligating the digested

DNAs to a vector comprising a promoter which is 5' to a recognition site for a second

restriction enzyme that generates 3' TA overhangs which is 5' to a recognition site for a

third restriction enzyme which generates blunt ends, which vector is digested with the

second and third restriction enzymes, to yield a plurality of mutagenized recombinant

vectors.

37. (Original) A method to prepare a plurality of mutagenized recombinant vectors,

comprising:

a) providing DNAs comprising a plurality of mutagenized open reading frames flanked

by a recognition site for a first restriction enzyme that generates a 3' TA overhang and site

for a second restriction enzyme which has infrequent restriction sites in cDNAs or open

reading frames from at least one species and generates blunt ends; and

b) digesting the DNAs with the first and second restriction enzymes and ligating the

digested DNAs to a vector comprising a promoter which is 5' to a SgfI recognition site

which is 5' to a recognition site for a third restriction enzyme which generates blunt ends,

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which vector is digested with SgfI and the third restriction enzyme, to yield a plurality of mutagenized recombinant vectors.

- 38. (Original) The method of claim 37 wherein the first restriction enzyme is *Pmel*.
- 39. (Original) A method to prepare a plurality of mutagenized recombinant vectors, comprising:
- a) providing DNAs comprising a plurality of mutagenized open reading frames flanked by two restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction enzyme and generates a first pair of non-self complementary single-strand DNA overhangs; and
- b) digesting the DNAs with the first restriction enzyme and ligating the digested DNAs to a vector comprising a promoter and non-essential DNA sequences flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic restriction enzyme, which vector is digested with the second restriction enzyme generating a DNA fragment which lacks non-essential DNA sequences but comprises a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs, to yield a plurality of mutagenized recombinant vectors.
- 40. (Original) A support comprising a plurality of mutagenized recombinant vectors prepared by the method of any one of claims 36 to 39.

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41. (Original) A library of recombinant cells comprising recombinant vectors, two or more of

which recombinant vectors comprise an open reading frame for a different polypeptide, wherein

at least one recombinant vector comprises a promoter and a first open reading frame which is

flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction

enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction

enzyme which generates blunt ends, which vector is digested with the first and second restriction

enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by

Sgfl and an end generated by a third restriction enzyme which has infrequent restriction sites in

cDNAs or open reading frames from at least one species and generates blunt ends.

42. (Original) A library of recombinant vectors, two or more of which recombinant vectors

comprise an open reading frame for a different polypeptide, wherein at least one recombinant

vector comprises a promoter and a first open reading frame which is flanked by two exchange

sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction

enzyme that generates 3' TA overhang which is 5' to a recognition site for a second restriction

enzyme which generates blunt ends, which vector is digested with the first and second restriction

enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by

SgfI and an end generated by a third restriction enzyme which has infrequent restriction sites in

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cDNAs or open reading frames from at least one species and generates blunt ends.

43. (Original) A library of recombinant cells comprising recombinant vectors, two or more of

which recombinant vectors comprise an open reading frame for a different polypeptide, wherein

at least one recombinant vector comprises a promoter and a first open reading frame comprising

a second open reading frame and one or more codons which are in-frame with the second open

reading frame, wherein the second open reading frame is flanked by two exchange sites, wherein

the exchange sites are formed by ligation of

a DNA sequence comprising the second open reading frame which includes a PmeI

recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that

generates complementary single-strand DNA overhangs, which DNA is digested with Pmel and

the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame

codons and the promoter which is 5' to an end generated by a second restriction enzyme which

generates single-strand DNA overhangs which are complementary to the single-strand DNA

overhangs generated by the first restriction enzyme.

44. (Original) A library of recombinant vectors, two or more of which recombinant vectors

comprise an open reading frame for a different polypeptide, wherein at least one recombinant

vector comprises a promoter and a first open reading frame comprising a second open reading

frame and one or more codons which are in-frame with the second open reading frame, wherein

the second open reading frame is flanked by two exchange sites, wherein the exchange sites are

formed by ligation of

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a DNA sequence comprising the second open reading frame which includes a PmeI recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA is digested with PmeI and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme.

- 45. (Original) A library of recombinant cells comprising recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of
- a DNA sequence comprising the open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the

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non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

46. (Original) A library of recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

47. (Original) A library of recombinant cells comprising recombinant vectors, a plurality of which comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a selected open reading frame, wherein at least one mutagenized recombinant vector

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comprises a promoter and a mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the mutagenized open reading frame flanked by an end generated by SgfI and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

48. (Original) A library of recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising a mutagenized open reading frames of a selected open reading frame, wherein at least one mutagenized recombinant vector comprises a promoter and a mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the mutagenized open reading frame flanked by an end generated by SgfI and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt

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ends.

49. (Original) A library of recombinant vectors, a plurality of which recombinant vectors

comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a

selected open reading frame, wherein at least one recombinant vector comprises a promoter and

an open reading frame comprising a mutagenized open reading frame and one or more codons

which are in-frame with the mutagenized open reading frame, wherein the mutagenized open

reading frame is flanked by two exchange sites, wherein the exchange sites are formed by

ligation of

a DNA sequence comprising the mutagenized open reading frame which includes a PmeI

recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that

generates complementary single-strand DNA overhangs, which DNA sequence is digested with

PmeI and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame

codons and the promoter which is 5' to an end generated by a second restriction enzyme which

generates single-strand DNA overhangs which are complementary to the single-strand DNA

overhangs generated by the first restriction enzyme.

50. (Original) A library of recombinant cells comprising recombinant vectors, a plurality of

which recombinant vectors comprise mutagenized recombinant vectors comprising mutagenized

open reading frames of a selected open reading frame, wherein at least one recombinant vector

comprises a promoter and an open reading frame comprising a mutagenized open reading frame

and one or more codons which are in-frame with the mutagenized open reading frame, wherein

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the mutagenized open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which includes a PmeI

recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that

generates complementary single-strand DNA overhangs, which DNA sequence is digested with

PmeI and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame

codons and the promoter which is 5' to an end generated by a second restriction enzyme which

generates single-strand DNA overhangs which are complementary to the single-strand DNA

overhangs generated by the first restriction enzyme.

51. (Original) A library of recombinant cells comprising recombinant vectors, a plurality of

which recombinant vectors comprise mutagenized recombinant vectors comprising open reading

frames of a selected open reading frame, wherein at least one recombinant vector comprises a

promoter operably linked to the mutagenized open reading frame which is flanked by two

exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which is flanked by at

least two restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic

restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate

a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA

overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by

two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic

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restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

52. (Original) A library of recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a selected open reading frame, wherein at least one recombinant vector comprises a promoter and the mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxoterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

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53. (Original) A method to introduce at least two recognition sites for at least two different restriction enzymes to the ends of an open reading frame, comprising:

- a) providing one or more nucleic acid sequences each comprising an open reading frame; and
- b) amplifying each nucleic acid sequence with at least a pair of oligonucleotides to yield amplified nucleic acid comprising sequences in the pair of oligonucleotides, wherein the pair of oligonucleotides has sequences which anneal to sequences in the one or more open reading frames, wherein the sequences in the amplified nucleic acid corresponding to sequences in one of the pair of oligonucleotides comprise a restriction enzyme site for *Sgf*I which is 5' to the sequences which anneal to the open reading frame, wherein the sequences in the amplified nucleic acid corresponding to sequences in the other of the pair comprises a restriction enzyme site for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, which first restriction enzyme site is 3' to the sequences which anneal to the open reading frame, and wherein the sequences in the amplified nucleic acid corresponding to sequences in the oligonucleotides are capable of being digested with *Sgf*I and the first restriction enzyme.
- 54. (Original) The method of claim 53 further comprising adding an adenine to the 3' ends of the amplified nucleic acid to yield a modified amplified nucleic acid fragment.
- 55. (Original) The method of claim 54 further comprising ligating the modified amplified nucleic acid fragment to a DNA fragment having a 5' T overhang to yield a recombinant vector.

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56. (Original) The method of claim 53 wherein the pair of oligonucleotides further comprise

a topoisomerase I binding site at the 5' end of the oligonucleotide.

57. (Original) The method of claim 56 further comprising ligating the amplified nucleic acid

fragment to a DNA fragment having blunt ends in the presence of topoisomerase I, to yield a

recombinant vector.

58. (Original) The method of claim 53 further comprising digesting the amplified nucleic

acid with Sgfl and the first restriction enzyme and ligating the digested amplified nucleic acid to

a DNA fragment having a blunt end and an end which is capable of ligation to an end generated

by SgfI, to yield a recombinant vector.

59. (Original) The method of claim 58 wherein the amplified nucleic acid is purified prior to

digestion.

60. (Original) The method of claim 58 wherein the amplified nucleic acid is purified after

digestion and prior to ligation.

61. (Original) The method of claim 53 wherein the nucleic acid sequence is cDNA.

62. (Original) The method of claim 53 wherein the nucleic acid sequence is RNA.

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63. (Original) The method of claim 53 wherein the one oligonucleotide of the pair which comprises the SgfI site includes an ATG 3' to the SgfI site which is in-frame with the open

reading frame.

64. (Original) The method of claim 53 wherein two or more different nucleic acid sequences

are amplified.

65. (Original) The method of claim 55, 57 or 58 further comprising transforming cells with

the recombinant vector to yield recombinant cells.

66. (Original) Recombinant cells prepared by the method of claim 65.

67. (Original) The library of any one of claims 41 to 42 and 47 to 48 wherein the at least one

recombinant vector comprises a further open reading frame flanked by two exchange sites,

wherein the exchange sites are formed by ligation of

the recombinant vector which comprises a recognition site for a fourth and a fifth

restriction enzyme site 3' to the recognition site for the restriction enzyme which generate blunt

ends, wherein the fourth restriction enzyme generates a 3' TA overhang and is different than the

first restriction enzyme, and wherein the fifth restriction enzyme generates blunt ends, which

vector is digested with the fourth and fifth restriction enzymes, and

a DNA sequence comprising the further open reading frame flanked by an end generated

by Sgfl and a sixth restriction enzyme which has infrequent restriction sites in cDNAs or open

reading frames from at least one species and generates blunt ends.